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(57) Abstract

The present invention relates generally to transgenic plants and is particularly directed to transgenic tuber plants and to DNA constructs useful for producing same. The DNA constructs comprise a first nucleotide sequence corresponding to a promoter capable of functioning in a plant and a second nucleotide sequence under control of said promoter and encoding a molecule capable of enhancing levels of a cytokinin in said plant.

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TRANSGENIC PLANTS

The present invention relates generally to transgenic plants and is particularly directed to transgenic tuber plants and to DNA constructs useful for producing same.

Tubers are a swollen part of a stem or root which are usually modified for storage. Many such tuber plants have assumed immense commercial importance in agriculture and horticulture. Important examples include potato, sugar beet, sweet potato, onion, garlic, artichoke and Dahlia.

Other plants, such as grasses and cereals, also use modified stems as important storage organs, particularly for storage of carbohydrate. These storage organs are important because they increase the capacity of the plant to sustain periods of stress through mobilisation of the stored carbohydrates and other compounds.

Stored reserves are also important in contributing to the grain yield of cereals (Borrell et al., 1989) and the field value of pasture grasses and legumes.

The potato is perhaps the best example of an economically important plant with a modified storage organ, in this case a tuber.

The potato is one of the world's most valuable food crops. The current production level of potatoes alone is estimated to be worth $US90 billion. By volume, potato ranks fourth in the world after rice, wheat and maize with about 300 million tonnes annually produced. Potato has been commercially produced in Europe and the USA for over 200 years, but it is a relatively new crop for many of the developing countries, although now, potato production is increasing in developing countries at a rate nearly twice that of most other food crops.

Tuber yield is a key determinant of the profitability of a tuber plant crop and yield is closely linked to tuber number and/or tuber weight on each plant. Tuber initiation is a function of genotype and various environmental conditions, but especially night temperature. Since night temperatures of less than 20°C are required for tuber initiation, tuber production, such as potato, in hot developing countries is restricted to elevated locations or the lowlands for short periods during the "cooler" part of the year.

Cytokinins have been implicated in the control of tuber initiation in plants such as potato. The ability to control the level of a cytokinin in such plants is, therefore, important for controlling tuberization.

The cloning and characterisation of the isopentenyl transferase (ipt) gene from Agrobacterium tumefaciens has led to the introduction of this gene into the DNA of plant cells. The unregulated production of cytokinins in tissues transformed with the ipt gene has typically resulted in complete inhibition of root formation (Smigocki and Owens, 1988). Recently, Medford et al. (1989) and Smigocki (1991) have reported rooted transgenic tobacco plants where the ipt gene was placed under the control of a heat-inducible promoter. In both these cases, although the effect of the inducers on gene expression was dramatic (i.e. remarkably elevated levels of cytokinin after heat treatment), the heat-inducible promoters appeared to be rather non-specific in that the plants exhibited phenotypes associated with excess cytokinin even without thermal induction.

There is a need, therefore, to utilise the benefits of the ipt or like gene in elevating cytokinin levels while not suppressing root formation. There is also a need to manipulate tuber plants, such as potato plants, to generate a transgenic plant with elevated cytokinin levels yet retaining at least normal root formation capability. In accordance with the present invention, there is provided a fusion between the chalcone synthase (chs) promoter from Antirrhinum majus and the ipt coding sequence. This construct can be used to

produce transgenic tuber plants with useful agronomic characteristics. In particular, the construct can be used to produce transgenic potato plants having improved tuber production. This type of construct is an example of a wider range of constructs which can be usefully employed in generating transgenic plants with improved properties.

Accordingly, one aspect of the present invention provides a DNA construct comprising a first nucleotide sequence corresponding to a promoter capable of functioning in a plant and a second nucleotide sequence under control of said promoter and encoding a molecule capable of enhancing levels of a cytokinin in said plant.

Preferably the plant is a tuber plant such as but not limited to potato, sugar beet, sweet potato, onion, garlic, artichoke or Dahlia. Most preferably the plant is potato.

The present invention is described and exemplified using the chs promoter from A. majus (Sommer and Saedler, 1986) and the ipt coding sequence (Barker et al., 1983; Heidekamp et al., 1983). This is done with the understanding, however, that the present invention extends to other functionally equivalent promoters or to other coding sequences which, like the ipt gene, result in elevated cytokinin levels. Sources of other genes that could act to increase levels of active cytokinins include Agrobacterium rhizogenes and Pseudomonas. Examples of alternative sources of promoters include genes such as those described in Keller et al. (1989) and Yang and Russell (1990) which are expressed in stem tissues. Reference herein, therefore, to the chs promoter and/or ipt gene or coding sequence is taken to include reference to other functionally similar promoters and/or genes.

Accordingly, in a preferred aspect of the present invention there is provided a DNA construct comprising a first nucleotide sequence corresponding to the chs promoter or a functional equivalent thereof and a second nucleotide sequence

under the control of said promoter encoding ipt or a functional equivalent thereof, wherein said DNA construct is capable of expressing said ipt gene or functional equivalent thereof in a tuber plant.

The first nucleotide sequence corresponding to the chs promoter may encode the entire naturally occurring promoter sequence or may contain single or multiple nucleotide substitutions, deletions and/or additions to the naturally occurring sequence provided such changes in nucleotide sequence result in a functional promoter. One skilled in the art will readily recognise that the promoter can be subject to a variety of mutational events and the effect of such events on the expression of sequences under the control of the promoter screened. Accordingly, reference herein to the chs promoter includes reference to the naturally occurring promoter and to any functional mutants or derivatives thereof. Examples of such mutants or derivatives include those resulting in constitutive expression, super-inducible expression and altering the control and/or inducibility of the promoter.

The second nucleotide sequence of the present invention is located "downstream" of the chs promoter and, hence, is under control thereof. Accordingly, the chs promoter directs transcription and ultimate expression of the second nucleotide sequence. In accordance with the present invention, the second nucleotide sequence corresponds to the naturally occurring ipt gene of A. tumefaciens or to any single or multiple nucleotide substitutions, additions and/or deletions, provided that any resulting mutant or derivative of the naturally occurring sequence encodes a polypeptide which is functionally similar to the ipt gene product and results in elevated cytokinin levels in a host plant. All altered but functional ipt genes are encompassed by the present invention. The present invention also extends to the recombinant product of the ipt gene or its mutants or derivatives when expressed by the chs or functionally equivalent promoter.

The first and second nucleotide sequences of the present invention are

referred to herein as a "DNA construct". The DNA construct of the present invention may exist alone or in combination with a larger DNA construct such as a vector molecule. Such a vector molecule may be replicable in prokaryotic and/or eukaryotic cells and may contain other coding promoter and/or regulatory sequences. Preferably, the vector is capable of facilitating entry of itself and/or a DNA construct into the genome of a plant cell.

In a most preferred embodiment, a promoter fragment from the A. majus chs gene is fused to the A. tumefaciens ipt gene to give plasmid pCGP275. The fusion is conveniently accomplished using in vitro mutagenesis to introduce a restriction site between the transcriptional and translational start sites of the chs gene. The introduction of the restriction site can be accomplished by a single or multiple base change but preferably only a single base is changed. In a most preferred embodiment, an XbaI site is introduced by a single base change (C→A) at position +35 of the chs gene.

Although the second nucleotide sequence of the present invention is directly controlled, i.e. expression directed, from the first nucleotide sequence, this expression may further be controlled by another regulatory sequence such as encoding a regulatory gene. Alternatively, the other nucleotide sequence may encode or be a cis controlling element. This is particularly useful if what is desired is to control developmentally the expression of the second nucleotide sequence or to induce expression during certain environmental conditions, climatic periods or certain months of the year. The stimulus to induce the overall expression may be environmental, developmental or may require the addition of external growth or other stimulatory factors.

Another aspect of the present invention is directed to a transgenic tuber plant carrying the DNA construct as hereinabove contemplated. More particularly, this aspect of the present invention provides a transgenic tuber plant carrying a DNA construct comprising a first nucleotide sequence corresponding to a promoter capable of functioning in said plant and a second nucleotide

sequence under the control of said promoter and encoding a molecule capable of enhancing levels of a cytokinin in said plant.

In a preferred aspect of the present invention there is provided a transgenic tuber plant carrying a DNA construct comprising a first nucleotide sequence corresponding to the chs promoter or a functional equivalent thereof and a second nucleotide sequence under the control of said promoter encoding ipt or a functional equivalent thereof, wherein said DNA construct is capable of expressing said ipt gene or functional equivalent thereof in said plant. Preferably, the tuber plant is potato, sugar beet, sweet potato, onion, garlic, artichoke or Dahlia. Most preferably, the tuber plant is potato.

The DNA construct may be introduced into the tuber plant via any number of convenient routes including mobilisation by Agrobacterium, transformation, microprojectile bombardment, micro-injection and electroporation of individual or groups of cells followed by plantlet regeneration.

Another aspect of the present invention contemplates a method for producing a transgenic tuber plant comprising preparing transgenic cells from a tuber plant carrying a DNA construct comprising a first nucleotide sequence corresponding to the chs promoter or functional equivalent thereof and a second nucleotide sequence under the control of said promoter encoding ipt or a functional equivalent thereof and then regenerating a tuber plant from said transgenic cells.

In accordance with the present invention, the transgenic tuber plants exhibit any one or more of the following properties: increased level of endogenous cytokinin(s); increased tuber number and/or weight; increased stem diameter; increased plant height; increased leaf size; delayed leaf senescence; increased photosynthetic capacity of leaves thereby increasing the ability of the plant to support an increased tuber load and hence increase tuber yield.

In addition, expression of the second nucleotide sequence of the DNA construct results in elevated cytokinin levels in stem tissue, such as developing stem tissue. Since stem tubers develop on the stem, the elevated cytokinin levels are directly implicated as causing at least an increase in number and/or weight of tubers per plant. However, expression need not be confined to the stem tissue and may also occur, for example, in leaves. Indeed, in some circumstances, it may be advantageous to have expression in leaves and stem to facilitate delayed leaf senescence as well as increased tuber initiation. The delayed leaf senescence may allow the plant to further support the increased tuber load and/or number.

Additionally, the insertion of the DNA construct of the present invention can lead to different transgenic effects which may result in different phenotypes. Although not intending to limit the present invention to any one theory as to mode of action, the different transgenic events may explain the varied properties (see above) observed in the transgenic plants. An additional property in some plants is the miniaturisation of tubers, such as observed in Figure 8. This may or may not be associated with an increase in number of tubers. Such miniature tubers may be important as specialised food items in the culinary arts.

The present invention is particularly described with reference to stem tubers. Examples of plants in this category include potato, onion, garlic and artichoke. However, the use of the term "stem tuber" should be construed in its broadest sense and include plants with modified stems such as bulbs, corms, rhizomes and cereals. Transgenic cereals having stems with increased thickness would be of value, for example, for wind resistance. In the floriculture industry, tulip, lily, freesia, gladiolus and Dahlia all have modified stems which are encompassed by the term "stem tubers" as used herein.

In accordance with the present invention, the transgenic potato plants produce an increased yield (i.e. weight and/or number) of tubers. Furthermore, when

potato tuberization occurs under non-inducing conditions, such transgenic potatoes will be very useful for cultivation in warmer climates such as developing countries.

The present invention also extends to tubers, such as potatoes, produced from the transgenic plants herein described.

The present invention is further described by reference to the following non-limiting Figures and Example.

In the Figures:

Figures 1(a) and (b) are a schematic representation of the introduction of an XbaI site at position +35 (indicated with *) of the chs sequence to form plasmid pCGP263.

Figures 2(a) and (b) are a schematic representation of the reconstruction of the chs promoter from plasmid pCPG263 to form plasmid pCGP267.

Figure 3 is a schematic representation showing the introduction of an XbaI site upstream of the ipt translation initiation codon.

Figures 4(a) and (b) are a schematic representation showing the formation of the chs-ipt fusion pCGP274.

Figure 5 is a schematic representation showing the Ti-binary construct pCGP275.

Figure 6 is a graphical representation showing the altered phenotypes of ten chs-ipt transgenic potato plants.

Figure 7 is a photographic representation showing increased tuber production

by potato plants 2-8, K-1 and K-2 transformed with pCGP275, compared to a non-transgenic control plant (W) at the same age.

Figure 8 is a photographic representation showing the production of a large number of small tubers (over 100) by a potato plant transformed with pCGP275 (K-4) compared to a non-transgenic control plant (W) of the same age.

Figure 9 shows photographic representations of transgenic potato plants transformed with pCGP275 compared to a non-transgenic control plant (W) of the same age:

a: shows the "shooty" spreading phenotype of K-4;

b: shows prolonged photosynthetic capacity (delayed leaf senescence) of K-2;

c: shows increased plant height and leaf size of K-1 and K-2.

Figure 10 is a photographic representation showing an autoradiograph of a Southern blot of potato DNA probed with a fragment of the ipt gene. Each lane contained 10µg DNA isolated from potato leaves, digested with EcoR1. C = untransformed control; 2-1 and K-4 = transgenic potato plants transformed with the pCGP275 construct. Filters were hybridized and washed under conditions of high stringency.

EXAMPLE

1. MATERIALS AND METHODS

Bacterial strains

The Agrobacterium tumefaciens strains used were K61 and LBA4404 (Hoekema et al., 1983).

The plasmid pCGP275 (Figure 5) was introduced into Agrobacterium by adding 5µg of DNA to 100µL of competent Agrobacterium cells. The

competent cells were prepared by inoculating 50mL of MG/L (Garfinkel and Nester, 1980) and growing for 16 hours with shaking at 28 °C. The cells were then pelleted and resuspended in 0.5mL of 85% (v/v) 100 mM $CaCl_2$/15% (v/v) glycerol. The DNA-Agrobacterium mixture was frozen by incubation in liquid N_2 for 2 minutes and then allowed to thaw for 5 minutes at 37°C. The DNA/bacterial mix was then placed on ice for a further 10 minutes. The cells were then mixed with 1mL of MG/L media and incubated with shaking for 16 hours at 28°C. Cells of A. tumefaciens carrying the plasmids were selected on MG/L agar plates containing 100 µg/mL gentamycin.

Potato transformation

Transformation of potato (cv. Desiree) leaf segments followed standard protocols and transformed plants were identified by their ability to grow on kanamycin-containing media.

Construction of pCGP275

A promoter fragment from the Antirrhinum majus chalcone synthase (chs) gene (Sommer and Saedler, 1986) was fused to the A. tumefaciens ipt gene (Barker et al., 1983; Heidekamp et al., 1983) to give pCGP275.

Construction of the fusions was facilitated using in vitro mutagenesis to introduce an XbaI site between the transcriptional and translational start sites of the chs gene. This was accomplished with a single base change (from C to A) at position +35 of the A. majus chs gene (Sommer and Saedler, 1986). Prior to mutagenesis, a small fragment of the chs 5' sequence was subcloned to avoid undesired mutations occurring at other sites in the chs sequence.

The various subcloning steps that preceded the mutagenesis are shown in Figures 1(a) and (b). A 5.7kb fragment containing 5' sequence from the A. majus chs gene was removed from plasmid AM3 (Wienand et al., 1982) by Eco RI digestion and inserted into the Eco RI site of pBluescript KS M13- (Stratagene). The resulting plasmid, designated pCGP253, was then digested

with ScaI and Eco RI and the 2.1kb fragment which included the chs initiation codon and 240bp of 5' untranslated leader sequence was ligated with a ScaI/Eco RI digest of pBR322. The resulting pBR322 derivative was designated pCGP260. The plasmid pCGP260 was digested with PstI and SacI and a fragment which included 235bp pBR322 sequence, the chs initiation codon and 240bp of untranslated leader sequence was ligated to a PstI/SacI digest of pBluescript KS M13- digested with the same enzymes to give pCGP262 (Figure 1b).

A XbaI site was introduced into pCGP262 at position +35 in the chs sequence (Sommer and Saedler, 1986) using the Biorad MUTAGENE kit and the synthetic oligonucleotide 5'-CAATCATCTAGAACAACCACTTC-3'. The modified plasmid was designated pCGP263 and the mutagenesis was confirmed by sequence analysis (Figure 1b).

The chs promoter was re-constructed as shown in Figures 2(a) and (b). The plasmid pCGP253 (shown in Figure 1a) was digested with Eco RI and the 5.7kb chs fragment inserted into the Eco RI site of pCGP263 to yield pCGP264. Plasmid pCGP264 contained the large chs promoter fragment in the same orientation as the mutated fragment (see Figure 2a). Plasmid pCGP264 was then partially restricted with ScaI and re-ligated. A plasmid which had lost the 2.1kb ScaI fragment was designated pCGP265 (Figure 2b). Plasmid pCGP265 was subsequently digested with AvaII and the overhanging 5' end filled in with the Klenow fragment of DNA Polymerase I prior to digestion with XbaI. The 1.2kb promoter fragment was isolated and inserted into pUC19 digested with SmaI and XbaI. The resulting plasmid was designated pCGP267.

chs-ipt Fusion

Plasmid pCGN1278 contained the ipt gene cloned into the SmaI site of pBluescript M13- as a RsaI fragment which included T-DNA from nucleotide 8487 to nucleotide 9836 (Barker et al., 1983). The ipt gene was removed from

pCGN1278 by digestion with Eco RI and XbaI, the overhanging 5' ends were filled in the Klenow fragment of DNA Polymerase I and the blunt-end fragment was inserted into the SmaI site of pBluescript KS M13-. The resulting plasmid was designated pCGP259.

Figure 3 shows the strategy used to introduce a XbaI site 8bp upstream of the ipt translation initiation codon. Plasmid pCGP259 was digested with BstXI and BspMI, ligated to the synthetic oligonucleotide 5'-AATTAGATGCAGGTCCATAAGTTTTTTCTAGACGCG-3' which included a XbaI site (underlined) and 5' and 3' ends complementary to the respective overhanging ends which remained after digestion of pCGP259. Following ligation, the single stranded gap was filled in using the Klenow fragment of DNA polymerase I. The modified ipt gene-containing plasmid was designated pCGP261 (Figure 3).

To construct the chs-ipt fusion, the chs promoter fragment was isolated from pCGP267 as a SacI/XbaI fragment and inserted upstream of the ipt gene in a SacI/XbaI digest of pCGP261 (Figure 4a). The SacI site in the resulting plasmid, pCGP273, was converted to a HindIII site by restriction with SacI, digestion of the overhanging 3' end with the Klenow fragment of DNA polymerase I and ligation of a HindIII linker to give pCGP274 (Figure 4b).

Ti-binary constructs

The HindIII fragment from pCGP274 containing the chs promoter fused to the ipt gene was inserted into the HindIII site of the binary vector pCGN1558 (McBride and Summerfelt, 1990) to give pCGP275 (Figure 5).

2. RESULTS

Generation of transgenic potato plants

Transgenic potato shoots carrying the chs-ipt gene fusion were selected on kanamycin-containing media after co-cultivation of leaf discs with either

K61/pCGP75 or LBA4404/pCGP275. Rooted shoots from ten separate transformation events were transferred to soil and grown to semi-maturity. Figure 6 summarises some of the phenotypic alterations that resulted from the introduction of the gene fusion. In all ten plants there is a significant increase in tuber yield compared to non-transgenic control plants. For the most part the yield increase is associated with an increase in the number of tubers initiated on the plants (Figures 6 and 7). One of the transgenic plants, K-4 (Figures 8 and 9a) was of particular interest in that it produced over one hundred small tubers. This plant had a more "shooty" spreading phenotype suggestive of decreased apical dominance, but it was able to root normally.

In plants other than K-4, the introduction of the chs-ipt fusion had the effect of increasing plant height and leaf size (Figures 6 and 9c). Most of the transgenic potato plants also had thicker stems than the non-transgenic control suggestive of preferential ipt expression in the stem tissues. The potato plants transformed with chs-ipt fusion also showed delayed leaf senescence (Figure 9b).

These data, therefore, indicate that potato plants transformed with the chs-ipt construct will be able to produce an increased and possibly high yield of tubers and may be able to be cultivated under non-inducing conditions in many of the warmer developing countries.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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CLAIMS:

1. A DNA construct comprising a first nucleotide sequence corresponding to a promoter capable of functioning in a plant and a second nucleotide sequence under control of said promoter and encoding a molecule capable of enhancing levels of a cytokinin in said plant.

2. The DNA construct according to claim 1 wherein the plant is a tuber plant.

3. The DNA construct according to claim 2 wherein the tuber plant is potato, sugar beet, sweet potato, onion, garlic, artichoke or Dahlia.

4. The DNA construct according to claim 3 wherein the tuber plant is potato.

5. The DNA construct according to claim 1 wherein the first nucleotide sequence corresponds to the chs promoter or to a functional equivalent thereof.

6. The DNA construct according to claim 1 wherein the second nucleotide sequence encodes ipt or a functional equivalent thereof.

7. A DNA construct comprising a first nucleotide sequence corresponding to the chs promoter or a functional equivalent thereof and a second nucleotide sequence under the control of said promoter encoding ipt or a functional equivalent thereof, wherein said DNA construct is capable of expressing said ipt gene or functional equivalent thereof in a tuber plant.

8. The DNA construct according to claim 7 wherein said tuber plant is potato, sugar beet, sweet potato, onion, garlic, artichoke or Dahlia.

9. The DNA construct according to claim 8 wherein the tuber plant is potato.

10. The DNA construct according to claim 1 or 5 wherein said construct is part of a larger DNA construct capable of being maintained in a prokaryotic and/or a eukaryotic cell.

11. The DNA construct according to claim 10 wherein the maintenance is either by integration into the cell genome or by autonomous replication.

12. The DNA construct according to claim 11 having the designation pCGP275, as hereinbefore described.

13. The DNA construct according to claim 11 wherein said promoter and/or said ipt gene or functional equivalents thereof is/are under the further control of a regulatory sequence.

14. The DNA construct according to claim 13 wherein the further control permits developmental regulation.

15. A transgenic tuber plant carrying a DNA construct comprising a first nucleotide sequence corresponding to a promoter capable of functioning in said plant and a second nucleotide sequence under the control of said promoter and encoding a molecule capable of enhancing levels of a cytokinin in said plant.

16. The transgenic tuber plant according to claim 15 wherein said plant is a potato, sugar beet, sweet potato, onion, garlic, artichoke or Dahlia.

17. The transgenic tuber plant according to claim 16 wherein said plant is potato.

18. The transgenic tuber plant according to claim 15 wherein the first nucleotide sequence corresponds to the chs promoter or to a functional equivalent thereof.

19. The transgenic tuber plant according to claim 15 wherein the second nucleotide sequence encodes ipt or a functional equivalent thereof.

20. A transgenic tuber plant carrying a DNA construct comprising a first nucleotide sequence corresponding to the chs promoter or a functional equivalent thereof and a second nucleotide sequence under the control of said promoter encoding ipt or a functional equivalent thereof, wherein said DNA construct is capable of expressing said ipt gene or functional equivalent thereof in said plant.

21. The transgenic tuber plant according to claim 20 wherein said plant is potato, sugar beet, sweet potato, onion, garlic, artichoke or Dahlia.

22. The transgenic tuber plant according to claim 21 wherein said plant is potato.

23. The transgenic tuber plant according to claim 15 or 20 wherein said promoter and/or said ipt gene or functional equivalents thereof is/are under the further control of a regulatory sequence.

24. The transgenic tuber plant according to claim 23 wherein the further control permits developmental regulation.

25. The transgenic tuber plant according to claim 15 or 20 exhibiting one or more of the following properties: increased level of endogenous cytokinin(s); increased tuber number and/or weight; increased stem diameter; increased plant height; increased leaf size; delayed leaf senescence; increased photosynthetic capacity of leaves thereby

increasing the ability of the plant to support an increased tuber load and increase tuber yield.

26. A transgenic tuber plant carrying a DNA construct corresponding in whole or in part to pCGP275, as hereinbefore described.

27. The transgenic tuber plant according to claim 26 wherein said plant is potato, sugar beet, sweet potato, onion, garlic, artichoke or Dahlia.

28. The transgenic tuber plant according to claim 27 wherein the transgenic tuber plant is potato.

29. A method for producing a transgenic tuber plant comprising preparing transgenic cells from a tuber plant carrying a DNA construct comprising a first nucleotide sequence corresponding to the chs promoter or functional equivalent thereof and a second nucleotide sequence under the control of said promoter encoding ipt or a functional equivalent thereof and then regenerating a tuber plant from said transgenic cells.

30. The method according to claim 29 wherein the tuber plant is potato, sugar beet, sweet potato, onion, garlic, artichoke or Dahlia.

31. The method according to claim 30 wherein the tuber plant is potato.

32. The method according to claim 29 wherein expression of said ipt or functional equivalent thereof is/are under the further control of a regulatory sequence.

33. The method according to claim 32 wherein the further control permits developmental regulation.

34. The method according to claim 29 wherein the transgenic cells are

prepared by mobilisation with Agrobacterium, transformations, microprojectile bombardment, micro-injection or electroporation.

35. The method according to claim 29 wherein the DNA construct is pCGP275.

36. The method according to claim 29 wherein the transgenic tuber plant is capable of tuberisation under non-inducing conditions.

37. The method according to claim 29 wherein said plant exhibits one or more of the following properties: increased level of endogenous cytokinin(s); increased tuber number and/or weight; increased stem diameter; increased plant height; increased leaf size; delayed leaf senescence; increased photosynthetic capacity of leaves thereby increasing the ability of the plant to support an increased tuber load and increase tuber yield.

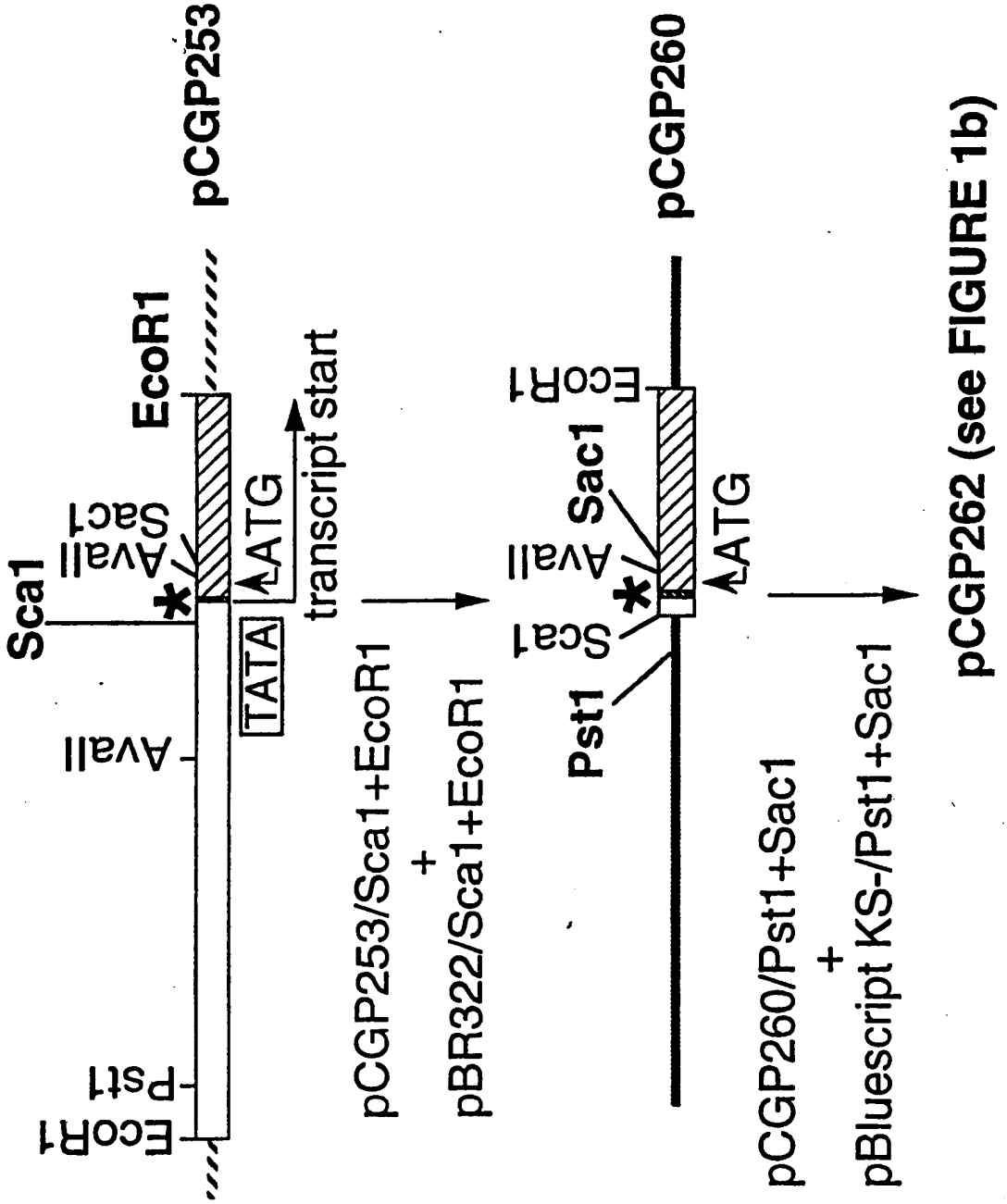
pCGP253
EcoR1
Sca1
ΔVaII
Sac1
ΔATG
TATA
transcript start
AvaII
PstI
EcoR1
pCGP253/Sca1+EcoR1
+
pBR322/Sca1+EcoR1
pCGP260
EcoR1
Sac1
AvaII
Sca1
ΔATG
Pst1
pCGP260/Pst1+Sac1
+
pBluescript KS-/Pst1+Sac1
pCGP262 (see FIGURE 1b)

FIGURE 1a

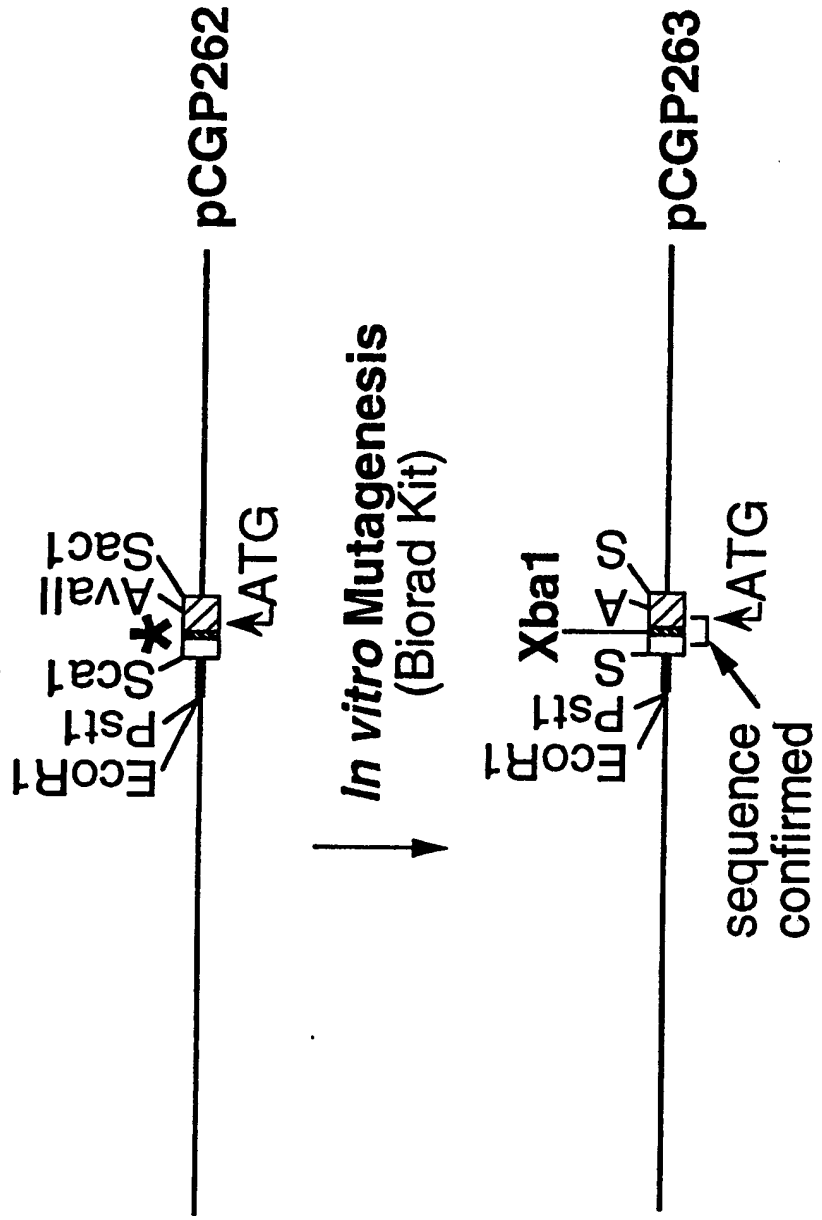
pCGP262
EcoR1
Pst1
Sca1
AvaII
Sac1
ATG
In vitro Mutagenesis
(Biorad Kit)
pCGP263
EcoR1
Pst1
S
A
Xba1
S
ATG
sequence
confirmed

FIGURE 1b

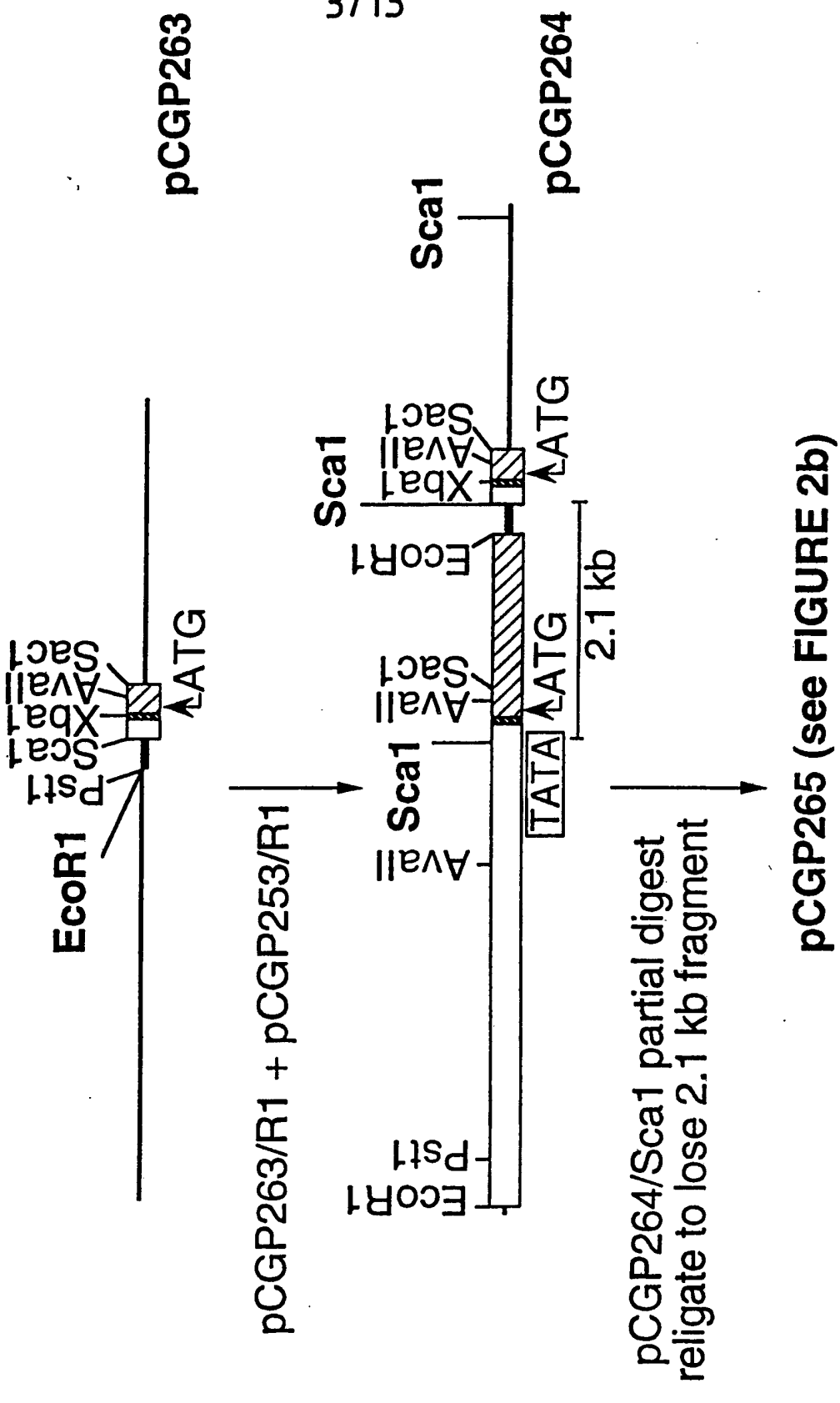
pCGP263
pCGP264
Sca1
Sca1
Sac1
AVaII
Xba1
ATG
EcoR1
2.1 kb
Sac1
AVaII
Xba1
Sca1
Pst1
EcoR1
ATG
TATA
AVaII
Sca1
EcoR1
Pst1
pCGP263/R1 + pCGP253/R1
pCGP264/Sca1 partial digest
religate to lose 2.1 kb fragment
pCGP265 (see FIGURE 2b)

FIGURE 2a

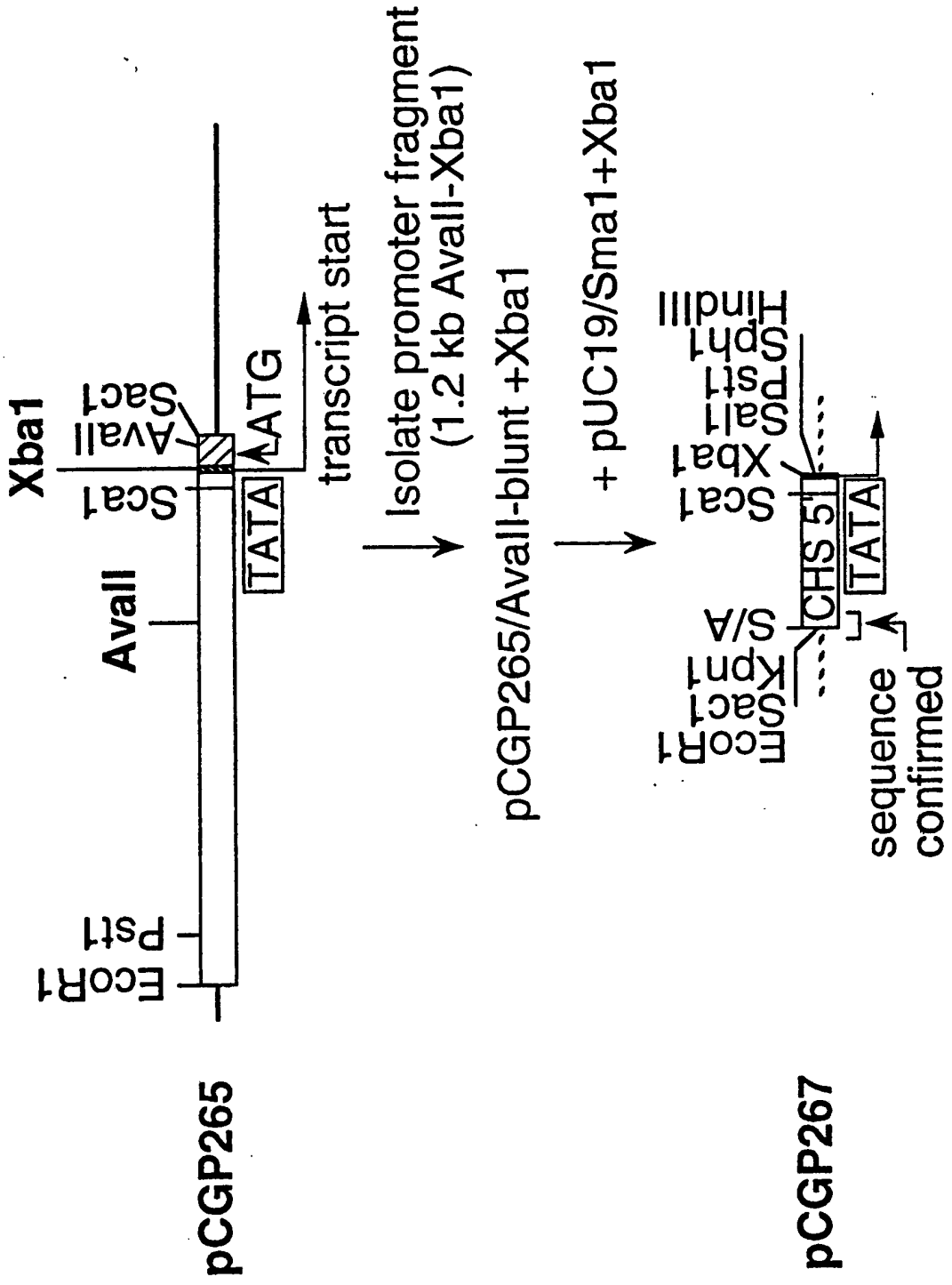
Xba1
AVaII
Sac1
Sca1
AvaII
TATA
ATG
transcript start
EcoR1
Pst1
pCGP265
Isolate promoter fragment
(1.2 kb AvaII-Xba1)
pCGP265/AvaII-blunt +Xba1
+ pUC19/Sma1+Xba1
HindIII
Sph1
Sst1
Sal1
Xba1
Sca1
CHS 5'
TATA
sequence
confirmed
pCGP267

FIGURE 2b

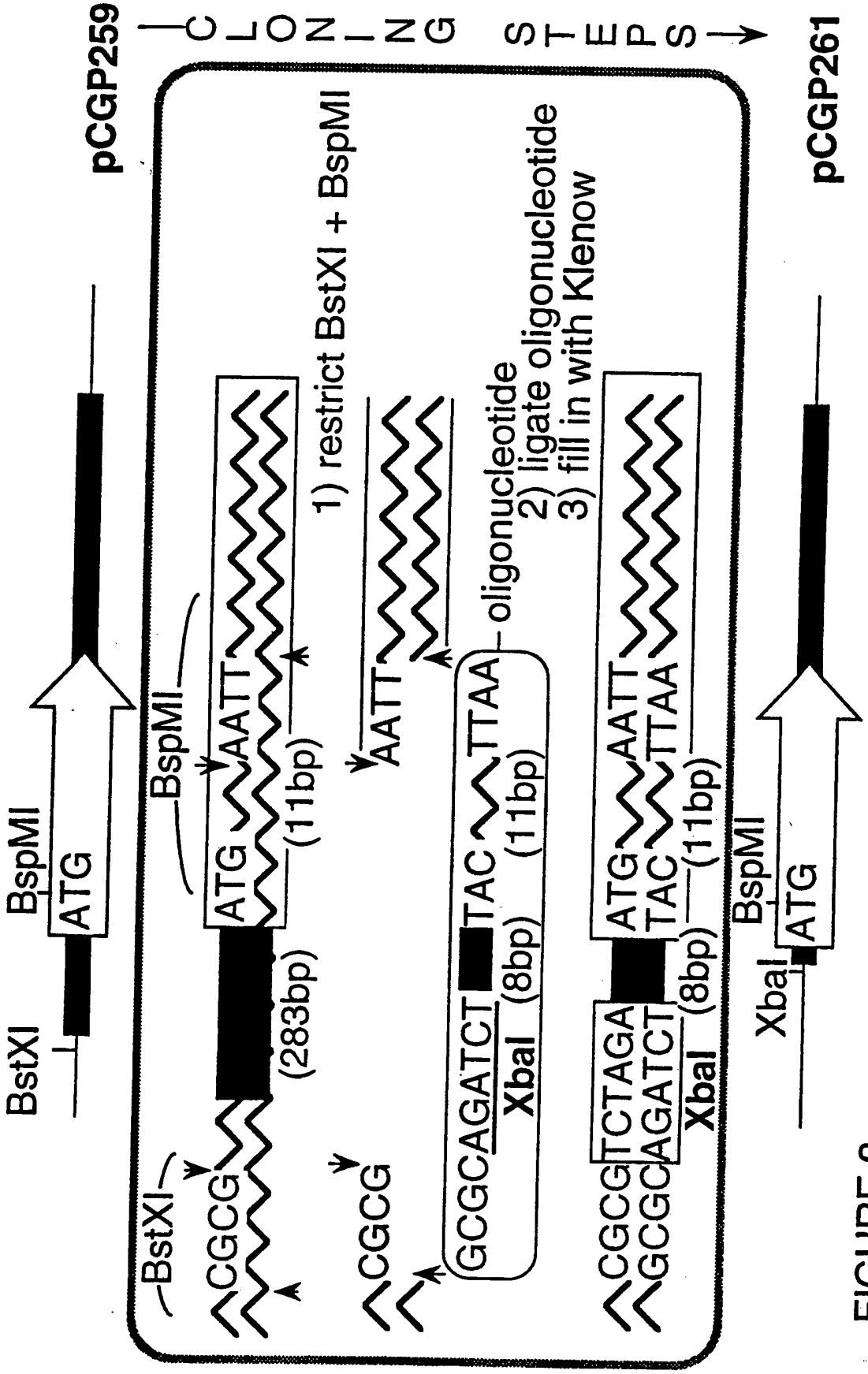

FIGURE 3

SUBSTITUTE SHEET

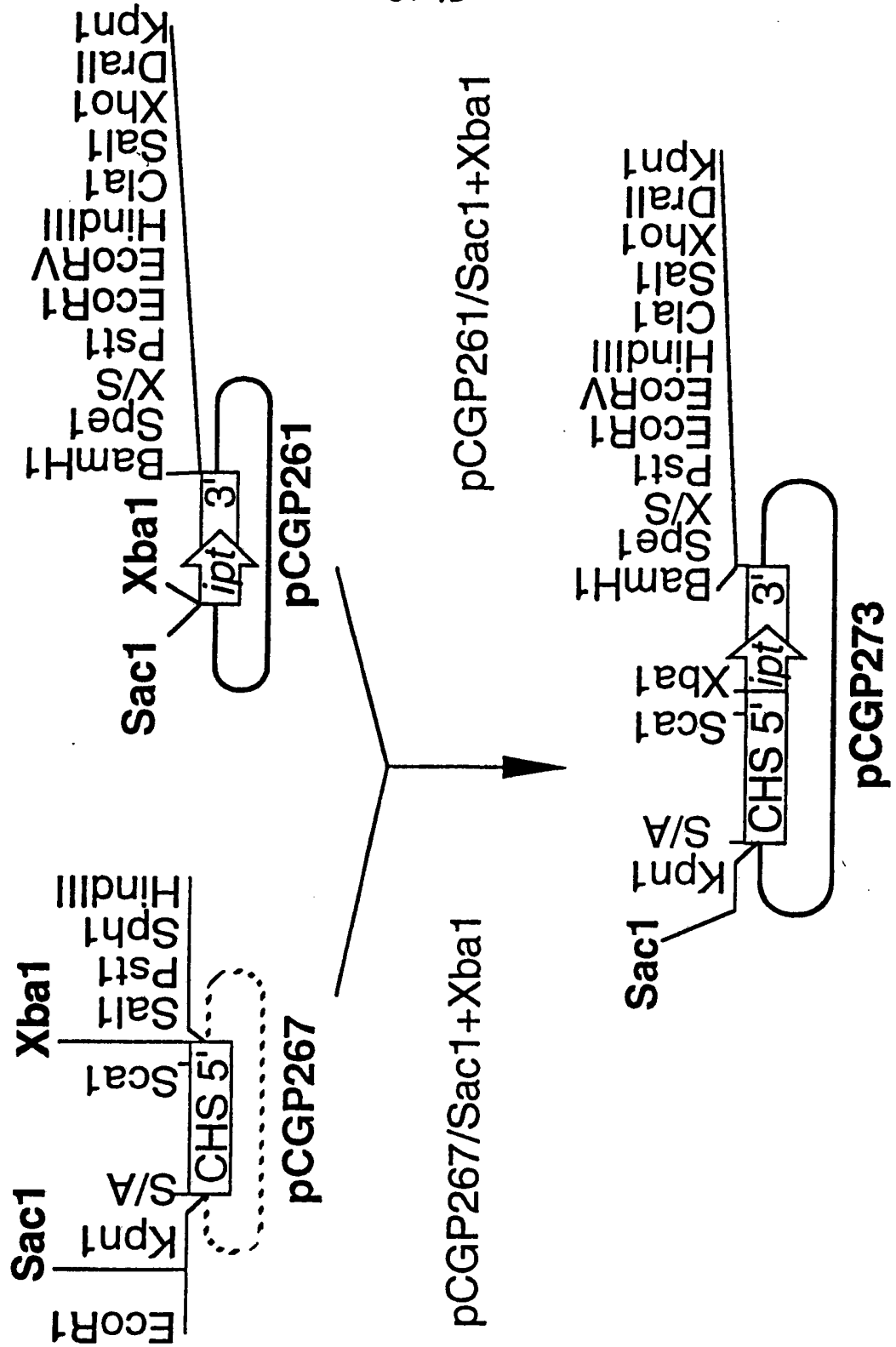
pCGP261
BamH1
Spe1
X/S
Pst1
EcoR1
EcoRV
HindIII
Cla1
Sal1
Xho1
DraII
Kpn1
Xba1
Sac1
3'
ipt
pCGP261/Sac1+Xba1
pCGP273
BamH1
Spe1
X/S
Pst1
EcoR1
EcoRV
HindIII
Cla1
Sal1
Xho1
DraII
Kpn1
Xba1
Sac1
3'
ipt
CHS 5'
Sca1
Kpn1
S/A
pCGP267
HindIII
Sph1
Pst1
Sal1
Xba1
Sca1
CHS 5'
S/A
Kpn1
Sac1
EcoR1
pCGP267/Sac1+Xba1

FIGURE 4a

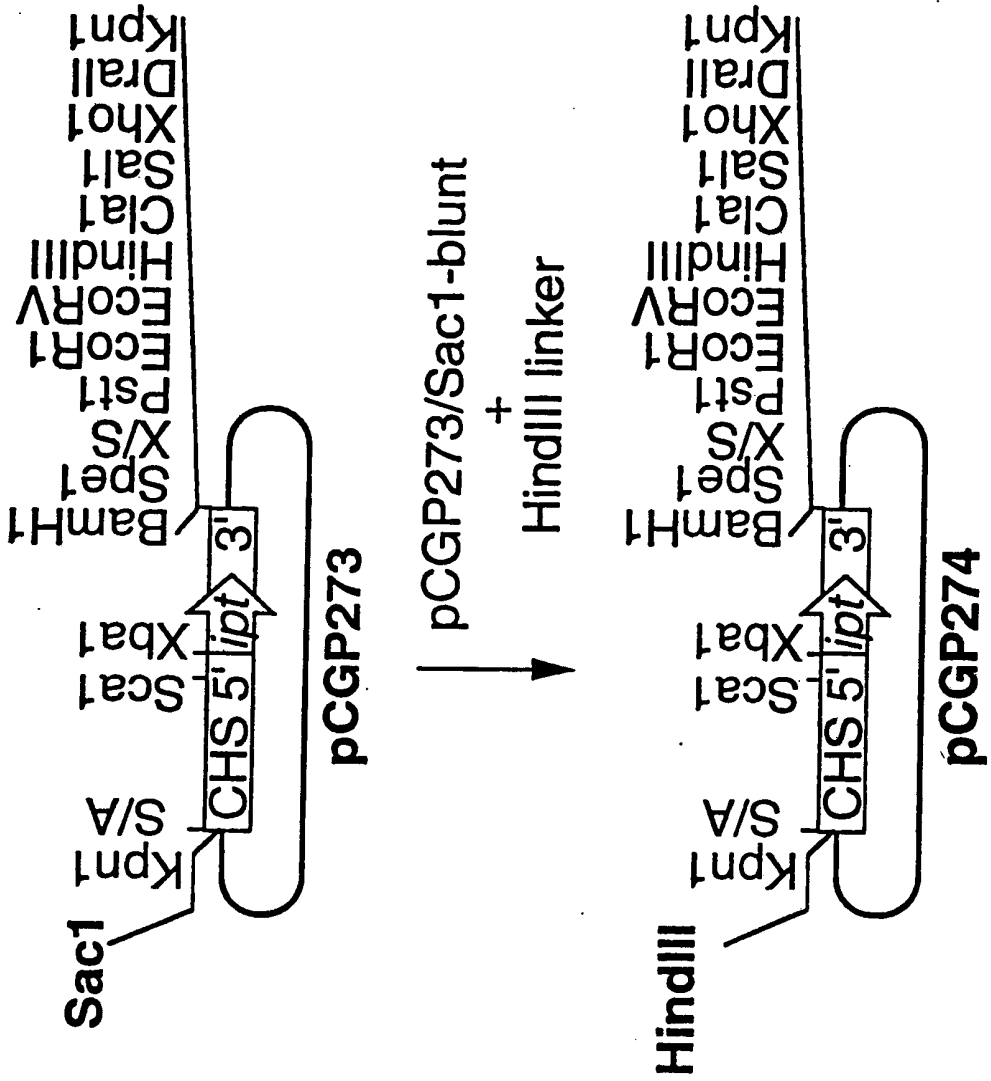
pCGP273
BamH1
Spe1
X/S
Pst1
EcoR1
EcoRV
HindIII
Cla1
Sal1
Xho1
DraII
Kpn1
3'
ipt
CHS 5'
Xba1
Sca1
S/A
Kpn1
Sac1
pCGP273/Sac1-blunt
+
HindIII linker
pCGP274
BamH1
Spe1
X/S
Pst1
EcoR1
EcoRV
HindIII
Cla1
Sal1
Xho1
DraII
Kpn1
3'
ipt
CHS 5'
Xba1
Sca1
S/A
Kpn1
HindIII

FIGURE 4b

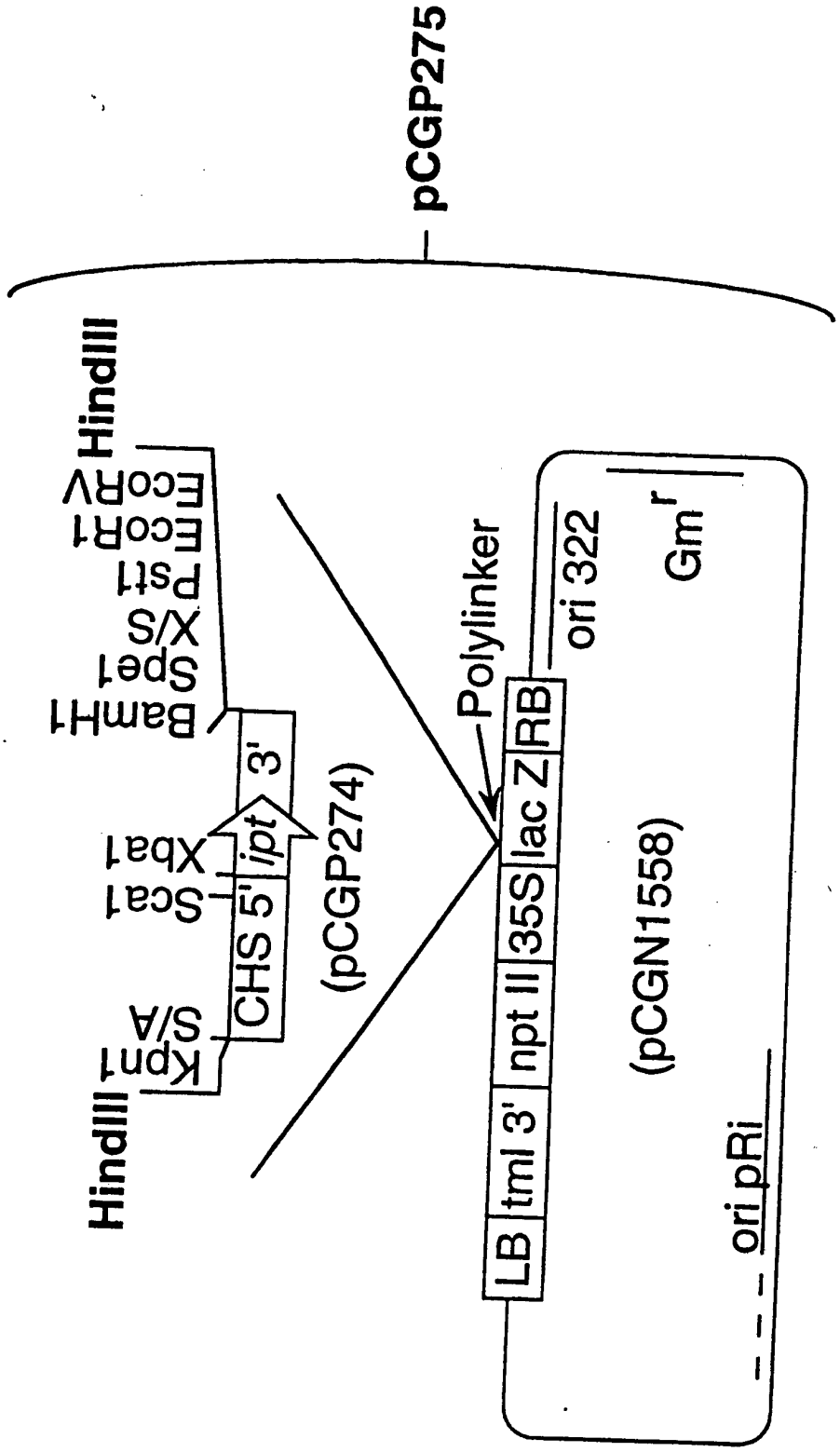
pCGP275
HindIII
EcoRV
EcoR1
Pst1
X/S
Spe1
BamH1
3'
ipt
CHS 5'
Sca1
Xba1
S/A
Kpn1
HindIII
(pCGP274)
Polylinker
RB
lac Z
35S
npt II
tml 3'
LB
ori 322
Gm^r
(pCGN1558)
ori pRi

FIGURE 5

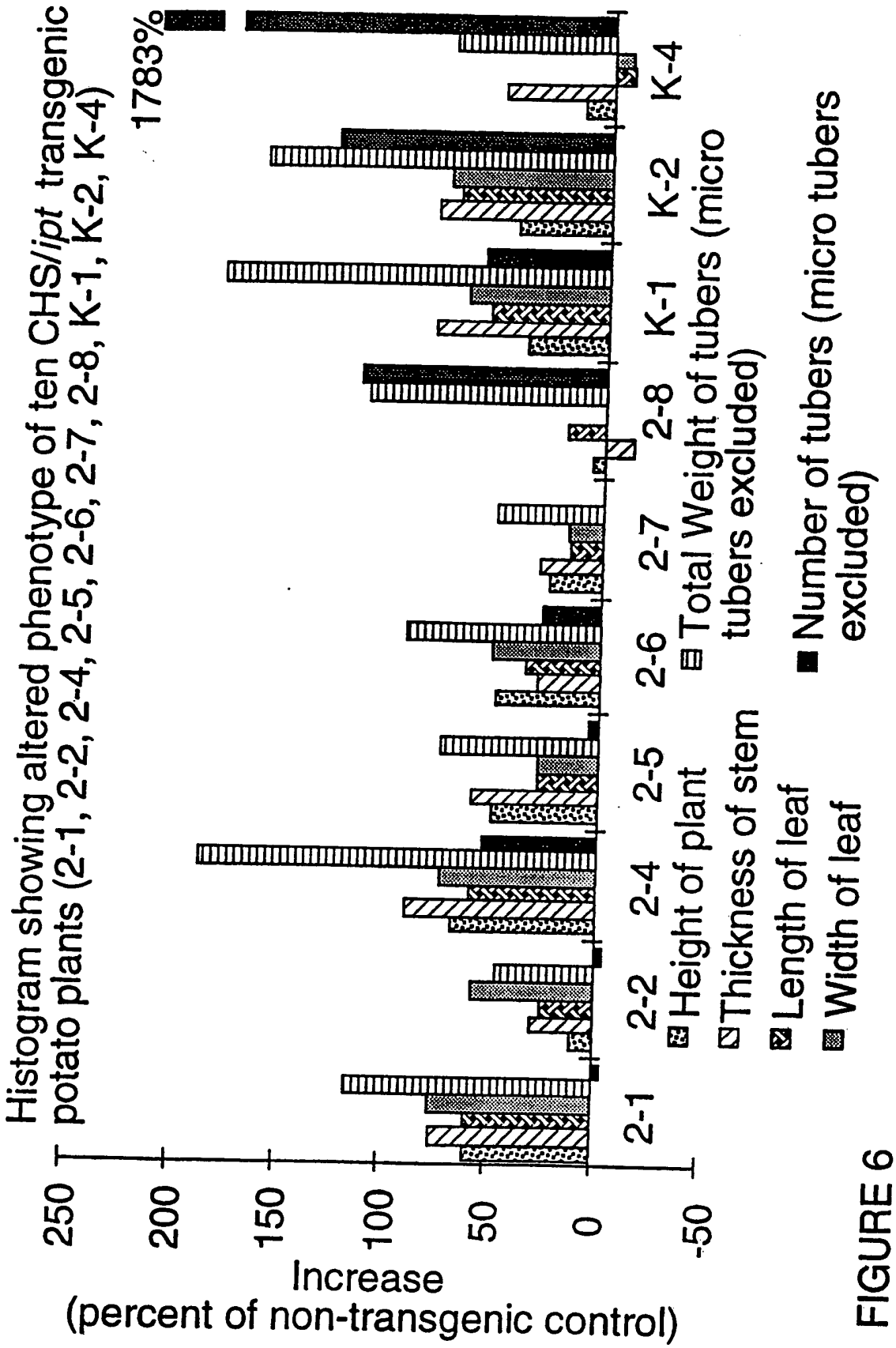
Histogram showing altered phenotype of ten CHS/ipt transgenic potato plants (2-1, 2-2, 2-4, 2-5, 2-6, 2-7, 2-8, K-1, K-2, K-4)
1783%
2-1
2-2
2-4
2-5
2-6
2-7
2-8
K-1
K-2
K-4
Height of plant
Thickness of stem
Length of leaf
Width of leaf
Total Weight of tubers (micro tubers excluded)
Number of tubers (micro tubers excluded)
250
200
150
100
50
0
-50
Increase
(percent of non-transgenic control)

FIGURE 6

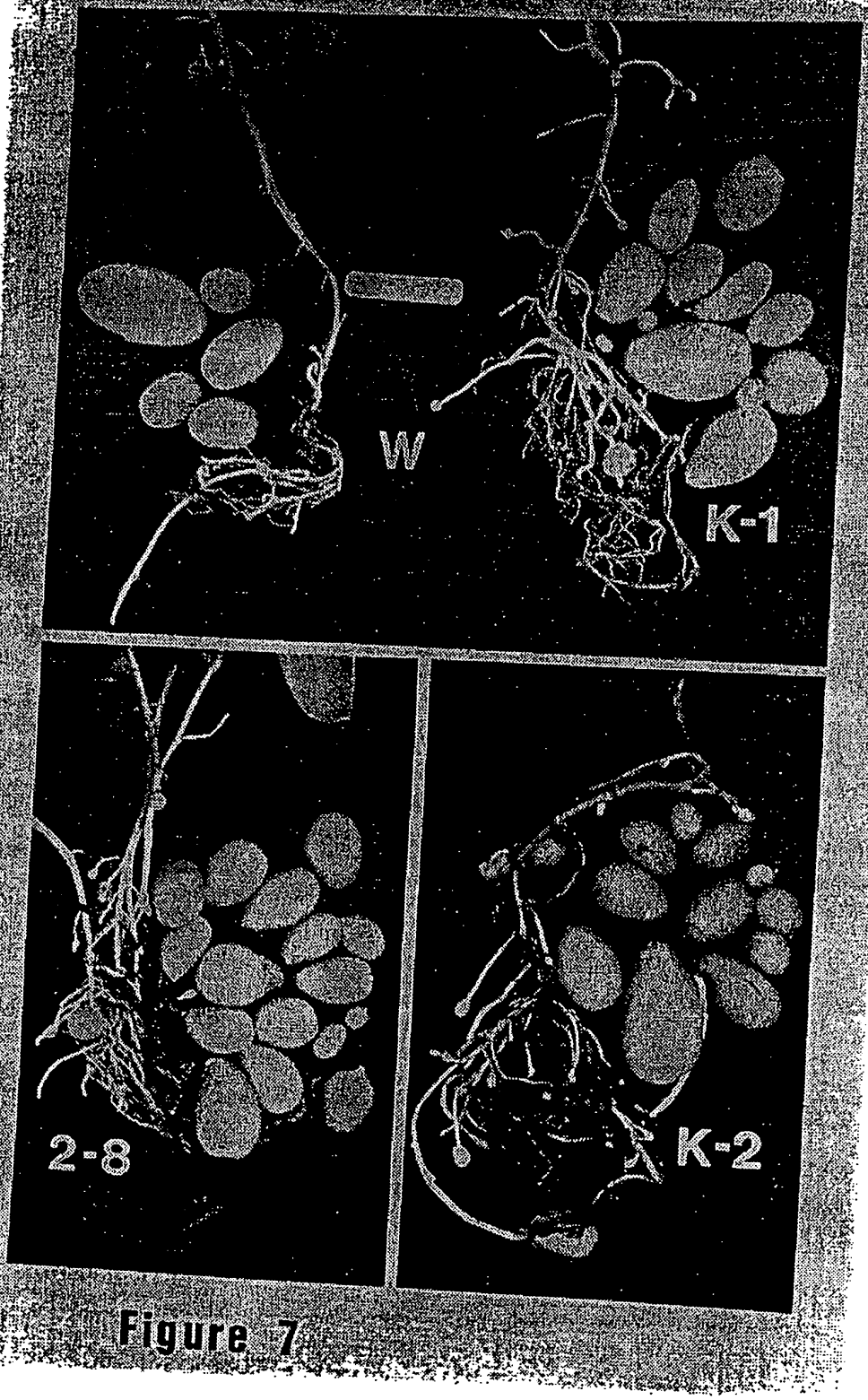

Figure 7

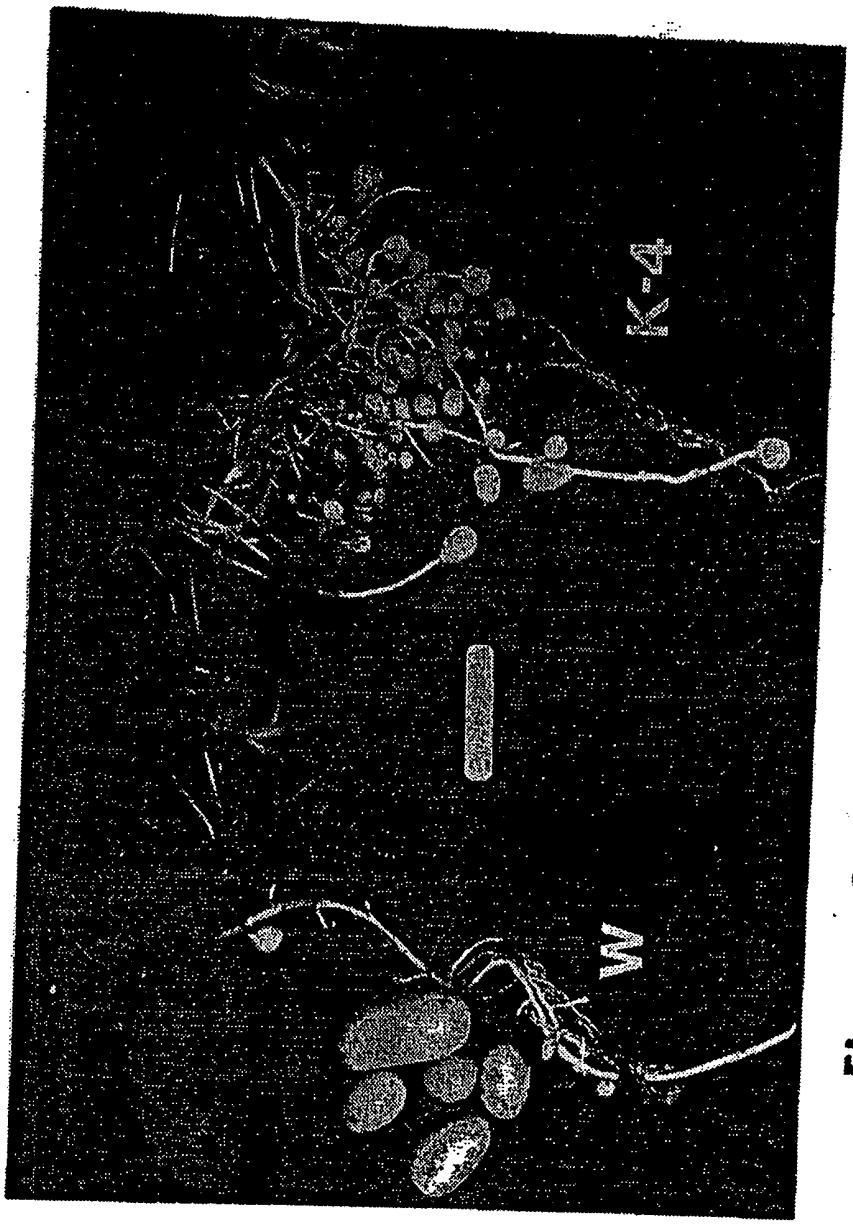

Figure 8

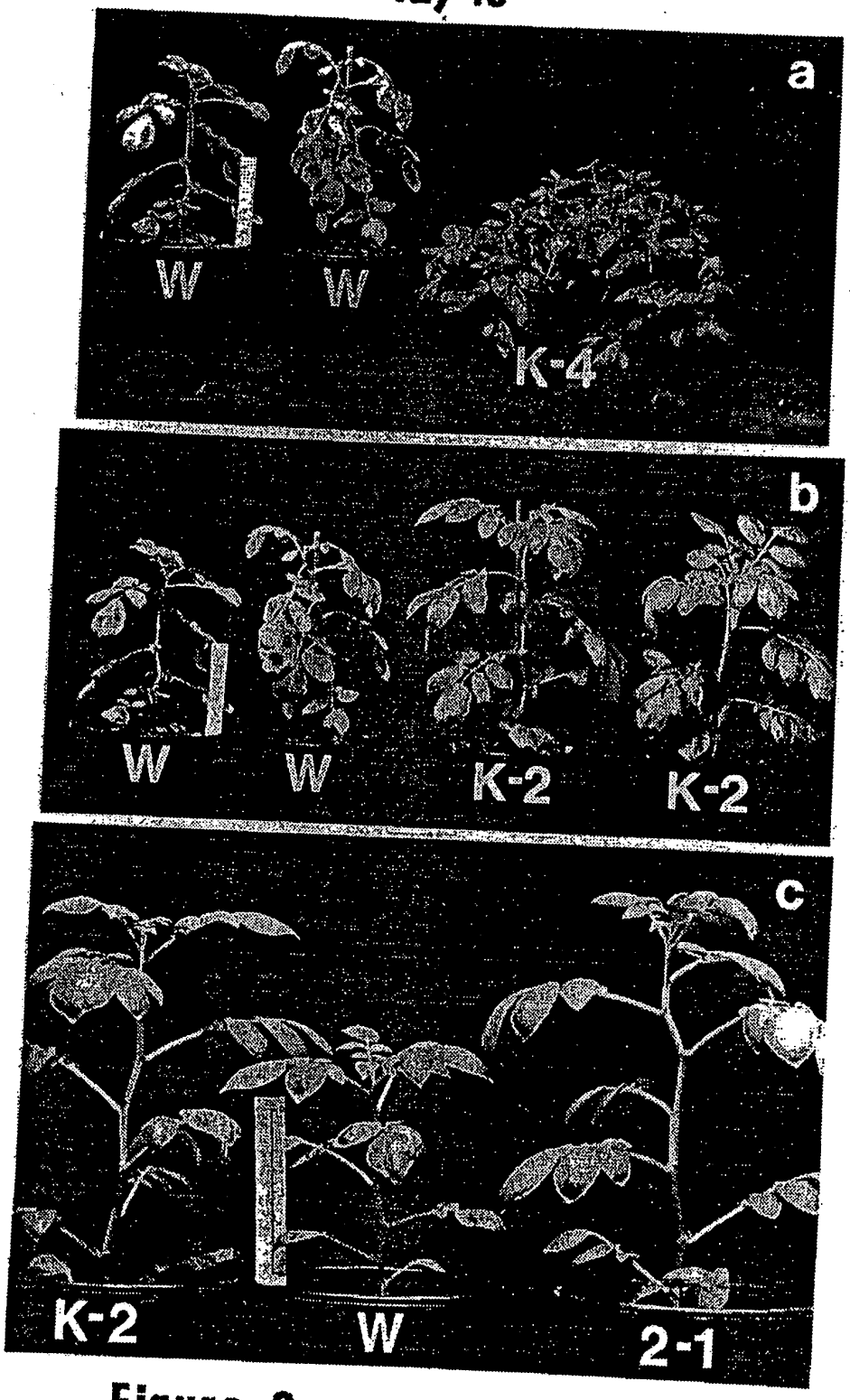

Figure 9

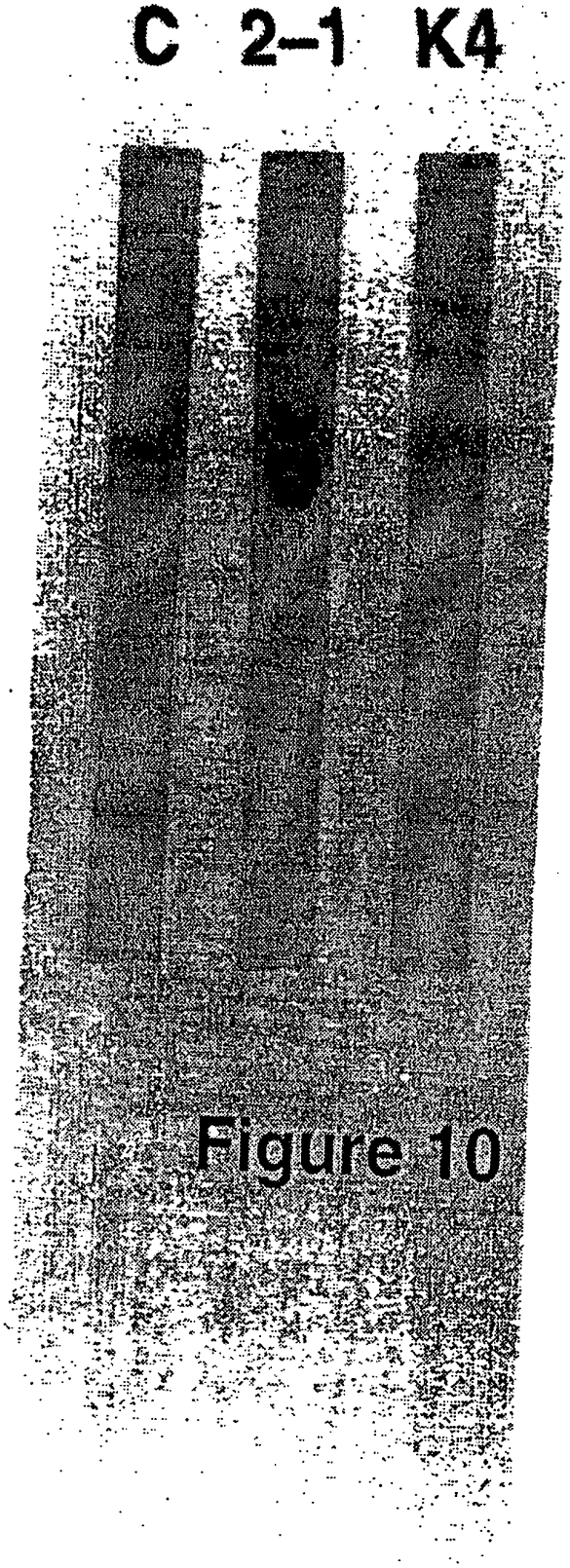

Figure 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU92/00528

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.[5] C12N 15/29, 15/54, 15/62, A01H 1/00, 5/00, 5/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC : A01H, C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU : C12N 15/29, 15/54, 15/62 A01H 1/00, 5/00, 5/06

Electronic data base consulted during the international search (name of data base, and where practicable, search terms used)
DERWENT DATA BASE; WPAT, BIOT, CHEMICAL ABSTRACTS DATA BASE, KEYWORDS - CYTOKININ, PLANT, DNA, CHALCONE SYNTHASE PROMOTER.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	WO,A, 91/01323 (CALGENE INC) 7 February 1991 (07.02.91) pages 10-13 claims 1-7	1, 5-6, 10-11, 13-14
X Y	FEBS Letters Volume 249 No. 2 June 1989 Schumulling T. et al "Construction of a heat inducible chimaeric gene to increase cytokinin content in a transgenic plant tissue" pp 401-406 whole article	1, 5-6, 10-11, 13-14 2-4, 7-9, 15-25, 27-34, 36-37

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 January 1993 (13.01.93)	19 JAN 1993 (19.01.93)
Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA	R. OSBORNE
Facsimile No. 06 2853929	Telephone No. (06) 2832111

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU92/00528

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate of the relevant passages	Relevant to Claim No.
Y	WO,A, 89/12059 (SALK) 14 December 1989 (14.12.89) claim 7	2-4, 7-9, 15-25, 27-34, 36-37
Y	Proc. Natl. Acad. Sci. Vol 87 June 1990, Yang N.S. et al "Maize sucrose-1 promoter directs phloem cell specific expression of Gus gene in transgenic tobacco plants" pp 4144-4148 whole article	2-4, 7-9, 15-25, 27-34, 36-37

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU92/00528

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member			
WO	91/1323	EP	409628	US	5177307
WO	89/12059	EP	414809		

END OF ANNEX